y the Triton X-100 cell lysates, soluble fraction. These fractions were pooled and dialyzed against three changes of water. The resulting supernatant was lyophilized and resuspended in PBS to a tenth of the original volume. This solution was exposed to crosslinking agents. The crosslinkers used were DSS (Pierce), and DTSSP (Pierce). The latter is cleavable with reducing agents, DSS is a nonreversible crosslinker. Both of these agents needed to be dissolved at 50 mg/ml, in a 50% V/V water: DMSO mixture.

The resulting crosslinked fractions were run on 12% SDS-PAGE, either a reducing, or a non-reducing gel, and analyzed by the multi-step western blot method. Nonreducing gels were identical to their reducing counterparts, except for the presence of 2-beta mercaptoethanol and DTT in the loading buffer. These were denaturing gels, so they did contain SDS.

Example 9

Mapping of the Vpr/Rip-1 Interaction

The approach used to determine the sites of this interaction was a peptide--blocking ELISA system. Briefly, rbp-1 was immobilized on ELISA plates (Immulon II, Dynatech Corp.), dissolved at approximately 1 .mu.g/ml in a 0.2M carbonate bicarbonate buffer, pH 9.2. The Vpr peptides were dissolved in blocking buffer at 50 .mu.g/ml, and incubated in the wells, using 50 .mu.l/well. These are overlapping peptides, which span the entire length of the Vpr molecule (obtained from the French AIDS Programme through the MRC repository, UK), the amino acid sequences of which are described in detail in Human Retroviruses and AIDS 1991, A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences, G. Myers et al., eds., Division of AIDS, National Institute of Allergy and Infectious Diseases, published by Theoretical Biology and Biophysics Group T-10, Los Alamos National Laboratory, Los Alamos, NM. Vpr was dissolved in blocking buffer at approximately 1 ug/ml. Different anti-Vpr antibodies were used to detect the amount of Vpr bound to the plates. Detection was accomplished by the use of a goat antiserum to mouse, or rabbit IgG, respectively, conjugated to horseradish, peroxidase.

In accordance with the above examples, it was found that the recombinant Vpr protein migrated predominantly as a putative monomer at 15 KD on SDS PAGE. The silver staining, and the western blot also revealed the presence of a possible homodimer at 30 KD, at a lower concentration than the monomer. This protein was identical to native protein in its SDS-PAGE migration characteristics as